New Zealand Journal of Botany, 1994, Vol. 32: 393–400
0028–825X/94/3204–0393 $2.50/0

Demography and conservation of *Botrychium australe*, a peculiar, sparse mycorrhizal fern

DAVE KELLY
Plant and Microbial Sciences
University of Canterbury
Private Bag 4800
Christchurch 1, New Zealand

Abstract *Botrychium australe* is a sparse native fern which is demographically unusual in having only a single leaf at any one time and in probably being strongly mycorrhizal. Individual plants of *B. australe* were mapped and followed at Cass, inland Canterbury, from 1987 to 1993. Plants were long-lived, with a mean half-life of 11.2 years. Some plants (21%) failed to produce any above-ground tissue in 1 or 2 years but later resumed normal growth. Up to 14% of plants were missing in this way in any one year. It is not known if this represents subterranean predation of fronds or quiescence while the fern is supported by mycorrhizae. Demographically, *B. australe* is more similar to some orchids than to other ferns. In any one year, only 9–20% of plants produced fertile spikes with sporangia. Fertile spike production was strongly correlated with higher light intensity; plants in deeper shade (<10% of full light) were very unlikely to be fertile. Plants in deeper shade also had larger sterile (green) fronds. The major identifiable cause of death was pig rooting, especially in 1989; over all years, 41% of deaths were attributed to pigs. *B. australe* appears to be detrimentally affected by shading by woody plants, and favoured by disturbance. Persistence of its populations may be enhanced by occasional disturbance (e.g., burning) where this does not conflict with other management aims.

Keywords *Botrychium australe*; Ophioglossaceae; demography; mycorrhizal associations; shade tolerance; orchids; life history; feral pigs; *Sus scrofa*

B94010
Received 4 February 1994; accepted 16 September 1994

INTRODUCTION

The family Ophioglossaceae in general, and the genus *Botrychium* in particular, are unusual within the ferns (in fact, Kato (1988) considers that the Ophioglossaceae are progymnosperms rather than ferns). Special features of the Ophioglossaceae include: very limited rates of frond production (usually only a single frond per year, so that the plant has only a single leaf at any one time); young fronds which do not emerge tightly rolled on themselves; the absence of root hairs; and often strongly developed mycorrhizal associations (Bower 1908, 1926; Bierhorst 1971; Braggins 1980; Montgomery 1990). Moreover, *Botrychium*, alone of all fern genera, also shows secondary thickening in the rhizome, and in some species contractile roots (Braggins 1980). These anatomical and architectural attributes mean that *Botrychium* species are demographically very unusual, resembling some orchids more than other ferns in several ways such as determinate adult plant size and partially heterotrophic nutrition. These features pose some difficulties for their management and conservation.

The most obvious way in which *Botrychium* species are unusual among plants (not just ferns) is the very deterministic growth form, where only a single frond is carried by the plant at any one time. The frond is rather unfernlike in appearance and comprises two parts: a sterile photosynthetic blade or lamina, and (optionally) a taller fertile spike which bears sporangia (Fig. 1). The fertile spike does not always develop, so only the sterile lamina is present on many plants. The spike separates from the stalk of the lamina below ground level, so it has the appearance of a separate frond, but it probably represents modified basal pinnae of a single frond, as shown by abberant fronds with a range of fertile and sterile parts and positions (Braggins 1969, 1980 fig. 6E, and my own observations at Cass). I will refer to the two parts as the lamina and spike, since they differ greatly in appearance and function. Braggins (1980) used the terms sterile lamina and fertile lamina. While the origin of the fertile segment

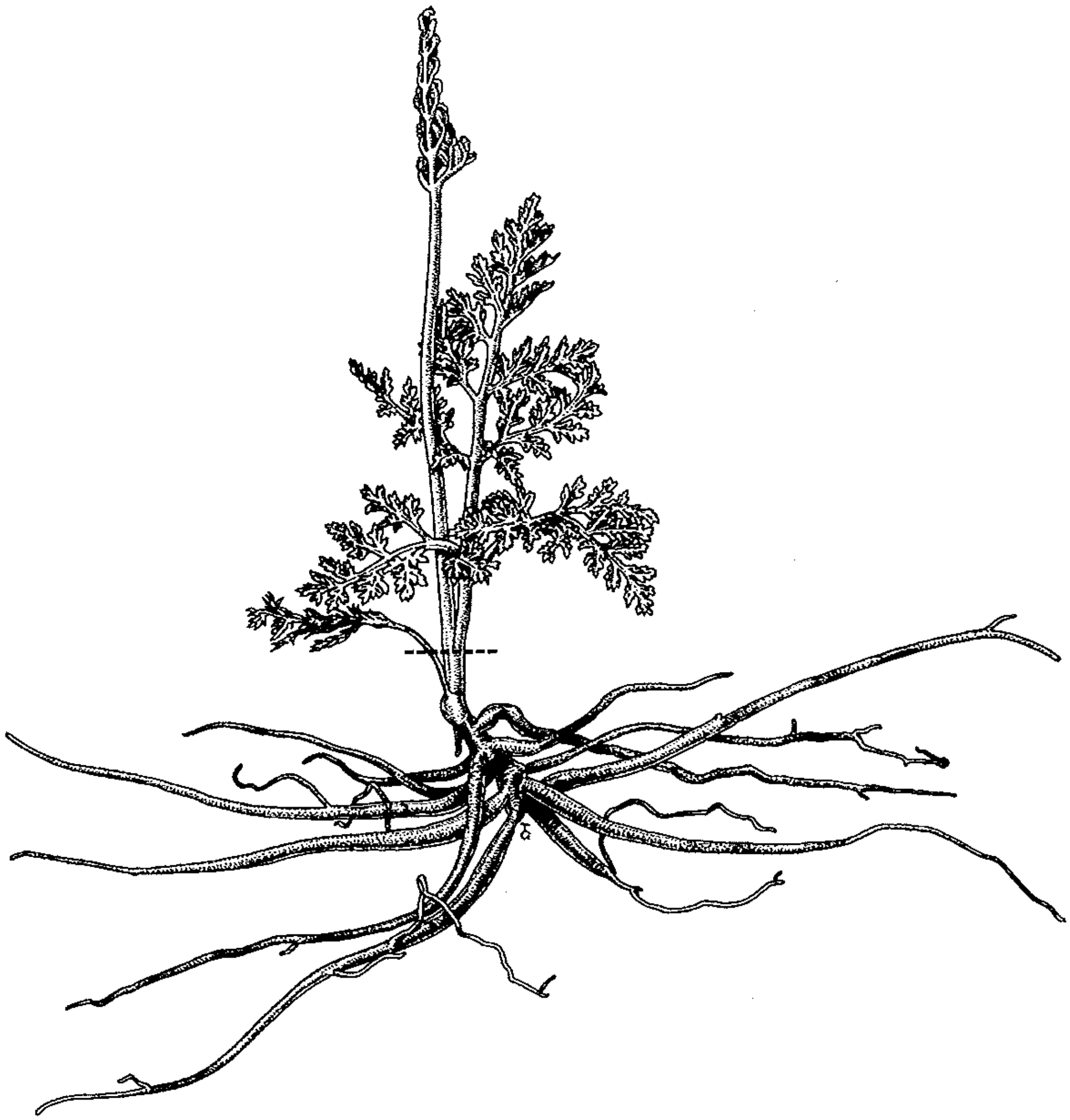

Fig. 1 Habit of *Botrychium australe*. The fertile spike (top) and sterile photosynthetic lamina (top right) are joined just at or below ground level (dotted line). The brown, wilted remains of last year's lamina is centre left. The short vertical rhizome bears numerous starch-filled contractile roots, which lack root hairs. Drawing by Tim Galloway from plants collected at Waterfall Terrace in May 1991 and August 1992.

is accurately described by that terminology, I do not follow it, since the fertile pinnae are so reduced that they bear little resemblance to a lamina.

There are three species of *Botrychium* in New Zealand (Braggins 1980; Brownsey et al. 1985; Brownsey & Smith-Dodsworth 1989). *B. lunaria* (L.) Swartz is morphologically very distinct, and extremely rare in New Zealand (Braggins 1980), and will not be considered further in this paper. *B. australe* R. Br. and *B. biforme* Col. are similar morphologically (*B. biforme* was previously treated as *B. australe* var *millefolium* Prantl.). The biology and morphology of the species were described in detail by Braggins (1980). Each species produces in

New Zealand a single frond in November–December, which lasts for 1 year. It dies down just before, or occasionally just after, the next season's frond is produced. The fertile spike is ephemeral, dying off soon after the spores are shed in autumn; it is initially green but later fades to yellow.

Botrychium species are also unusual underground (Braggins 1980). The plants have a short, vertical rhizome, 1–5 cm long, giving rise to 10–30 lateral roots. The rhizome is slow growing and produces secondary vascular tissue, but rarely, if ever, branches (Bierhorst 1971; Braggins 1980). The roots are thick and fleshy, storing large amounts of starch; in *B. australe* the roots are also contractile, pulling the rhizome down into the ground. The roots lack root hairs, though the total length of roots averaged 4 m in *B. biforme* and 6 m in *B. australe* (Braggins 1980).

Another feature of the Ophioglossaceae is that they are generally mycorrhizal, especially in *Botrychium* where this has been shown for at least 12 species including *B. lunaria* (Bower 1908, 1926; Bierhorst 1971). The mycorrhizae probably provide much of the absorbing surface (Bierhorst 1971), especially given the absence of root hairs. The gametophytes of *Botrychium* are subterranean and wholly dependent on mycorrhizae for their nutrition. The sporophytes are also intially dependent on the fungal association for up to 8 or 9 years before producing their first leaf above-ground (Bower 1908; Bierhorst 1971; Braggins 1980; Mason & Farrar 1989), although in axenic culture some *Botrychium* species may produce their first leaf within a year of fertilisation (Whittier & Thomas 1993). In this respect, the *Botrychium* life cycle again shows many similarities to that seen in orchids (Montgomery 1990).

Both *B. australe* and *B. biforme* are widespread but sparse in New Zealand, although they are inconspicuous and may have been under-reported (Braggins 1980). Both species may be locally dense, occurring in disjoint patches scattered through both the North and South Islands. Therefore, while not threatened (cf. Wilson & Given 1989), they are noteworthy plants from a conservation point of view. Although their taxonomy and morphology have been well described by Braggins (1980), relatively little is known of their demography and ecology. Demographic data may be of great assistance in protecting or enhancing populations of these species.

This study therefore had two aims: (1) to gather data on how the very unusual growth form of these plants affects their survival and reproduction; and (2) to document the demographic and ecological features of the plants, which might be relevant to their conservation.

MATERIALS AND METHODS

The study population of *Botrychium australe* is on Waterfall Terrace (grid reference NZMS 260, K34/066966) on Grasmere Station, near Cass, inland Canterbury. This population has been long studied; it is the "Cass" population for which chromosome counts are provided by Brownlie (1958; J. D. Lovis pers. comm.) and which is mapped in Braggins (1980, fig. 4). The site is bisected by the stream draining Horrible Bog and is at an altitude of 630 m. The soils are Bealey series (weakly podsolised upland/high country yellow-brown earths; Cutler 1977). The vegetation at the site is dense shrubs with scattered areas of introduced grasses and some patches of bare soil. The main shrub species are *Discaria toumatou*, *Coprosma propinqua*, *C.* sp. aff. *parviflora* ('sp. T'), *Leptospermum scoparium*, *Cassinia leptophylla*, and *Aristotelia fruticosa*; the most common ground cover species are *Agrostis tenuis*, *Anthoxanthum odoratum*, *Lycopodium fastigiatum*, *Blechnum penna-marina*, *Pteridium esculentum*, and *Leucopogon fraseri*. Over the period of this study, the area was only lightly grazed by cattle, and there was a noticeable increase in the cover of all shrub species at the expense of grass and open space. A voucher specimen of *Botrychium australe* is deposited in the University of Canterbury herbarium (CANU 35074).

At the site, 20 wooden pegs were hammered into the ground near *B. australe* plants, and the fronds around the pegs were mapped using distance and direction from the peg. Each year the site was re-mapped from 1987 to 1994 in early May (June in 1992), and the mapped plants were searched for within a distance of ±1 cm from the previous year's frond. If a plant was not seen for 2–3 years consecutively it was deemed to have died. New plants appearing close to the pegs were added to the records, but were not systematically searched for. Therefore, the starting sample size for survival over each year varied continuously with deaths and additions to the records.

The locations of plants, and of extensive pig rooting which occurred in 1989, were mapped to calculate the area turned over by pigs.

Each located plant had the size of its lamina (length × width) in the natural attitude in the field recorded, along with any description of evident grazing or damage. The area covered by the triangular lamina was estimated as (length × width × 0.5). Where a plant was fertile, the total height of the spike and the length of its spore-bearing part were recorded as indices of reproductive output.

The light intensity at ground level beside each plant was recorded in August–October 1993 for all plants seen at least since 1990. Two Campbell CR21X dataloggers with LiCor LI190 quantum sensors were taken to the site on an overcast day. One recorded the light intensity in the open, while the other was used for spot readings at the location of each plant. The reading next to each plant was then expressed as a percentage of the open reading at that moment. Readings on overcast days are relatively unresponsive to directionality of canopy openings, even though more sunlight would enter a northerly canopy opening. However, this will vary daily and seasonally with the movement of the sun, and can only be quantified with hemispherical photographs or long-term use of light meters. For instantaneous readings, overcast conditions provide the best indication of average light intensity.

To examine the patterns of underground branching, a patch of six *Botrychium* plants near the study area was excavated in August 1993. The roots were carefully removed to search for any connections between plants, or evidence of horizontal rhizome growth.

RESULTS

Plant form

The *B. australe* plants generally carried only a single sterile lamina per year. Occasionally a frond would branch just above or just below ground level, so that the lamina was divided into two parts. Examination of underground tissues showed no evidence of interconnection between adjacent plants, either by horizontal stem growth, or by grafting or adventitious shoots on the roots. One plant was found to have one large and one small frond emerging from opposite sides of the top, apparently a result of mechanical damage to the top of the stem. Therefore, *B. australe* seems to have little or no potential for vegetative spread, with each plant remaining in one spot and producing only a single leaf per year.

Survival and half-life

B. australe plants had high survival between years, with survival from one year to the next usually being >90% (Table 1). Cumulative survival after 6 years was 69%, giving a half-life for the whole population of 11.2 years. However, this varied widely among years. There was high mortality in 1988/89, which was partly attributed to extensive pig rooting in the study area (see below). For the 4 years (1990–93) after this period of high mortality, the half-life of the population was 19.9 years, and only a single plant seems to have died in 1993. Even this single death will not be confirmed until 1995. Some plants which were thought to be dead would later reappear, having failed to produce a leaf in some years, so the distinction between dead plants and those temporarily absent above-ground cannot be made until several years later. These data have been corrected for reappearances until May 1994.

Missing plants

Occasionally, a plant would be relocated, but the whole of the leaf had been eaten or broken off, leaving only a stump. In other cases, the plant did not produce any evident leaf in 1 year; the remains of the previous year's lamina would sometimes be found as confirmation that the correct spot was being searched. It is not known whether this lack of a leaf represents cryptic (subterranean) leaf predation, or simply quiescence on the part of the plant in failing to produce a leaf in that year.

Therefore, "missing" plants were defined as those where no sign was seen of a leaf in a particular year, but in following years the plant was once again found. The fraction of plants that were missing

Table 1 Survival and reproduction rates for marked plants of *Botrychium australe* at Cass, inland Canterbury, 1987–93.

Year	Survival (%)	n	Cumulative survival	Half-life (yr)	Fertile (%)	Missing (%)
1987	nd	64	100	nd	27	nd
1988	90.6	88	90.6	7.1	11	5
1989	87.5	112	79.3	5.2	19	12
1990	97.0	112	76.9	22.8	12	1
1991	92.9	117	71.4	9.4	15	14
1992	97.4	116	69.6	26.3	9	4
1993*	99.1	na	69.0	80.0	11	2

*Corrected for two missing plants which reappeared in 1994 census.

n = the number of mapped plants alive each year which were followed until the next year; nd, no data; na, not applicable.

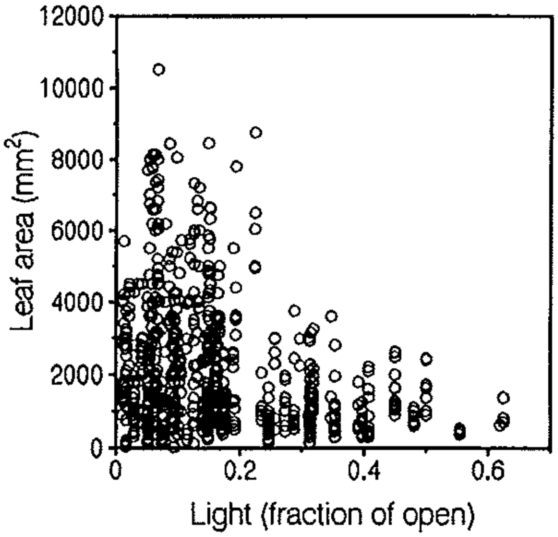

Fig. 2 Leaf "area" (length × width × 0.5) of *Botrychium australe* plants at Waterfall Terrace, 1987–93, versus light intensity by each plant (fraction of full light).

varied widely between years, from 1% in 1990, to 14% in 1991 (Table 1). Over all possible cases (the 128 plants seen for at least 3 years), 79% of plants did not miss any years, 16% missed a single year at some time, 3% (four plants) missed 2 years consecutively then reappeared, and 2% (two plants) missed 2 non-consecutive years.

Pig rooting

Feral pigs (*Sus scrofa*) are present in the study area and signs of old pig rooting are widespread. In May 1989, extensive recent pig rooting had turned over the soil on c. 22% of the study area. At first this was thought to have seriously depleted the *B. australe* population, as 16% of the marked plants (14 plants) grew in areas of pig rooting and failed to appear. However, in the subsequent 2 years, six of the 14 plants reappeared, making the likely mortality in 1989 due to pigs 9.3%. There was occasional pig rooting in other years, and over the whole study period 13 of 32 deaths of plants (41%) occurred immediately following pig rooting at that spot.

Leaf area versus light

Most *Botrychium* plants were growing either around the edges of shrubs or in the shade underneath them. The highest light intensity recorded for a mapped plant was 62% of full light, but most plants were in <20% (Table 2), and the most shaded plant was in just 1% of full light.

The size of the lamina varied widely, with most leaves being <4000 mm^2 (100 × 80 mm; Fig. 2). The largest lamina seen was 10 500 mm^2 (150 × 140 mm), although Braggins (1980) reported leaves up to 250 × 250 mm. At the other extreme, some plants would consistently produce leaves of only 50 mm^2 (10 × 10 mm). There was a strong relationship with light intensity (Fig. 2), so that plants at high light intensities always had small leaves, whereas in more shady areas plants could have small to large leaves. In general, the leaves in the shade were also thinner and brighter green; leaves in bright light tended to have a bronzed or brown colour (see also Braggins 1980), although this was not universal. From Fig. 2 it seems that in this population the upper limit of leaf size was reached when light intensity fell to about 20% of full light, below which plants were unable to increase leaf area any further to compensate for decreasing light intensities. Since the size of the current leaf represents the entire photosynthetic area of the plant in this species, it was assumed that, in a given light intensity, larger leaved plants were more vigorous and better established.

Reproduction

Most of the plants produced a fertile spike only rarely or not at all (Table 2). Disregarding the first year, for which the data may be biased because fertile plants are more conspicuous, <20% of plants were fertile in any year (Table 1). There was variation from year to year in the fertile fraction of the population, but this was not significantly correlated with the fraction of plants missing in each year (R^2 = 0.776, n = 6, P = 0.070), although curiously in 1989 and 1991, both variables were high simultaneously.

Fertile spikes versus light

The chance that a plant would be fertile at some time over the study interval was strongly affected by its light environment. Significantly more of the plants in very open areas were fertile at some stage (Table 2; χ^2 = 18.67, d.f. = 3, P < 0.001). Some of the plants

Table 2 Percentage of *Botrychium australe* plants fertile at any time over the study period, in areas of different light intensity (% of open).

Light (%)	n	% ever fertile
<10	51	18
10–20	42	36
20–40	32	31
>40	9	89

in very open areas produced fertile spikes in most years (e.g., one plant in 50% of full light was fertile in 1987, 1988, 1989, 1992, and 1993). In contrast, plants in deep shade (<10%) very rarely produced fertile spikes at all. There was a significant relationship between light intensity at each plant and the fraction of years that it was fertile ($y = -0.0043 + 0.816x$, $n = 134$, $F = 31.9$, $P < 0.001$, $R^2 = 0.195$).

DISCUSSION

Growth form: similarities to terrestrial orchids

These field observations on *B. australe* agree with previous work on *Botrychium* showing that plants produce only a single leaf per year and do not spread vegetatively by rhizomes (Bierhorst 1971; Braggins 1980; Montgomery 1990). This unusual habit means that the single above-ground leaf represents the entire size of the photosynthetic surface for that complete year. It is not at all clear whether there are advantages to such a constrained growth form, which is particularly serious if that one leaf is damaged by herbivory, since a damaged leaf is not replaced before the following spring. It seems remarkable that such a small herbaceous plant can survive the absence of green leaves for so long (up to 2 consecutive years in this study). This has also been shown for *Botrychium dissectum* in the U.S.A., where in a 12 year study Montgomery (1990) found that many plants were eaten off early in the season (for up to 9 consecutive years) without dying, and many other plants were missing for 1 year or more.

That the plant can cope with such damage, and also with failing entirely to produce a leaf in some years, is presumably due to two factors. Firstly, the fleshy roots contain much starch in larger plants (Braggins 1980). The bud at the top of the rhizome already contains the leaves which will expand over the next 4 or 5 years (Braggins 1980; Montgomery 1990), so the plant could draw on its reserves to survive the leafless period and expand the new leaf next year. However, it is not clear why the next leaf is not produced early following damage to the current leaf. Braggins (1980, p. 361) showed that precocious leaf expansion can occur, but only following exposure of the stem apex to light rather than damage to the current leaf.

Secondly, the possibility of saprophytic nutrition via mycorrhizae means that the *Botrychium* plants may not be totally dependent on their own photosynthate. In this respect, they are similar to orchids (Montgomery 1990), which have also been shown to frequently not produce leaves above ground in some years (e.g., Wells 1967; Summerhayes 1968). Overall, the similarities between the life cycles of terrestrial orchids and *Botrychium* species are marked: very small size of the disseminule (seed or spore), heterotrophic nutrition of the developing plant underground for a number of years, long-lived adult plants, few small above-ground parts (e.g., a single leaf per year) produced by large fleshy storage organs in the roots, and ability to survive years with no above-ground tissues.

Population dynamics: what habitat suits *Botrychium*?

The ecology of *B. australe* is still not completely understood. Firstly, the present study does not document the process of establishment of new sporophyte plants. This would be problematic to study, since the gametophyte is subterranean, and new sporophytes may live heterotrophically underground for anywhere between 1 and 9 years before producing their first visible leaf (Bower 1908; Bierhorst 1971; Braggins 1980; Mason & Farrar 1989; Whittier & Thomas 1993).

However, certain conclusions can be drawn from the existing data. *B. australe* plants in deep shade rarely produce fertile spikes, as reported also by Braggins (1980), and hence are unable to contribute much to the next generation of gametophytes. The plants in <10% of full light generally did not seem to be doing very well, even though they made up nearly half of the population at Waterfall Terrace. Braggins (1980) stated that *B. australe* was less shade tolerant than *B. biforme*. Few plants in this study were growing in very exposed situations, but this could result from a wide range of possible causes. There may be a higher probability of herbivory or trampling damage by cattle in more open places; the microclimate may be less favourable in open areas; or competition with grasses may be detrimental to *B. australe*. Nevertheless, at Waterfall Terrace, the few open-grown plants are currently responsible for most of the spore production in the population.

Over the study period, there was an increase in the cover of shrubs at this site. Therefore, the *B. australe* population might best be viewed as pioneer, hanging on in the ever-deepening shade, but increasingly less able to reproduce. Throughout New Zealand, *B. australe* and *B. biforme* are reportedly associated with disturbance, and are found in habitats such as river flats, track edges, and reverting pasture

(Braggins 1980; Brownsey & Smith-Dodsworth 1989). The plants would be well equipped to resist above-ground disturbance such as fire (Braggins 1980), with their large starch reserves in the roots, their ability to survive the removal of the current lamina, and (in *B. australe*) contractile roots which pull the rhizome down below the soil surface.

Conservation and management

The population of *B. australe* at Waterfall Terrace has been there since at least the mid 1950s, but currently conditions there do not seem to suit it very well. If it was thought desirable to preserve or increase the population, two actions could be taken. The first would be reducing numbers of feral pigs, since these were responsible for nearly half the deaths of plants over the study period. The 11.2 year half-life of plants during 1987–93 is much shorter than the 35.7 years for *B. dissectum* at less disturbed sites in the U.S.A. (calculated from data in Montgomery 1990). Although comparisons between such different habitats and species are difficult, the short half-life at Cass implies that a high rate of establishment of new sporophytes would be required if the population is not to disappear. Secondly, limiting the expansion of woody vegetation by some kind of managed disturbance, such as fire or brushcutting, may provide locations for colonisation by new *Botrychium* plants, without killing all the adult sporophytes (although this suggestion will remain speculative until data are available on gametophyte establishment in *Botrychium*). Pig rooting may provide opportunities for colonisation, but only at the cost of deaths of adult plants. Burning or cutting the native shrubs at the site may conflict with other conservation or farming aims for the area, but the section containing *B. australe* appears to be only a small part of the whole terrace, and has been so for at least the last 35 years (J. D. Lovis pers. comm.). However, there is much more research required before we will fully understand the biology of this fascinating and peculiar plant.

ACKNOWLEDGMENTS

I thank Colin Burrows for first showing me this population of *B. australe*; the runholders of Grasmere Station for permission to work on Waterfall Terrace; Lynn Adams, Mark Bachels, Marian Dawn, Richard Griffiths, Bronwyn Jones, Jane Marshall, Paul Millthorpe, Jon Sullivan, and the students of PAMS 204 (1987–93) for help with fieldwork; Tim Galloway for the drawing of *B. australe*; and Mike Hutchings, Janice Lord, John Lovis, Ashley Sparrow, and Jon Sullivan for comments on the draft.

REFERENCES

Bierhorst, D. W. 1971: Morphology of vascular plants. New York, Macmillan.

Bower, F. O. 1908: The origin of a land flora. London, Macmillan & Co.

Bower, F. O. 1926: The ferns (Filicales), Vol. 2. Cambridge, Cambridge University Press.

Braggins, J. E. 1969: The New Zealand species of *Botrychium* Swartz. Unpublished M.Sc. thesis, lodged in the Library, Victoria University of Wellington.

Braggins, J. E. 1980: Some studies on the New Zealand species of *Botrychium* Sw. (Ophioglossaceae). *New Zealand journal of botany 18*: 353–366.

Brownlie, G. 1958: Chromosome numbers in New Zealand ferns. *Transactions of the Royal Society of New Zealand 85*: 213–216.

Brownsey, P. J.; Smith-Dodsworth, J. C. 1989: New Zealand ferns and allied plants. Auckland, David Bateman.

Brownsey, P. J.; Given, D. R.; Lovis, J. D. 1985: A revised classification of New Zealand pteridophytes with a synonymic checklist of species. *New Zealand journal of botany 23*: 431–489.

Cutler, E. J. 1977: Soils of the Cass District. *In*: Burrows, C. J. *ed*. Cass: history and science in the Cass district, Canterbury, New Zealand. Christchurch, Department of Botany, University of Canterbury. Pp. 117–141.

Kato, M. 1988: The phylogenetic relationship of Ophioglossaceae. *Taxon 37*: 381–386.

Mason, N. A.; Farrar, D. R. 1989: Recovery of *Botrychium* gametophytes, gemmae, and immature sporophytes by centrifugation. *American fern journal 79*: 143–145.

Montgomery, J. D. 1990: Survivorship and predation changes in five populations of *Botrychium dissectum* in eastern Pennsylvania. *American fern journal 80*: 173–182.

Summerhayes, V. S. 1968: Wild orchids of Britain. 2nd ed. London, Collins.

Wells, T. C. E. 1967: Changes in a population of *Spiranthes spiralis* (L.) Chevall. at Knocking Hoe National Nature Reserve, Bedfordshire. *Journal of ecology 55*: 83–99.

Whittier, D. P.; Thomas, R. D. 1993: Gametophytes and young sporophytes of *Botrychium jenmanii* in axenic culture. *International journal of plant sciences 154*: 68–74.

Wilson, C. M.; Given, D. R. 1989: Threatened plants of New Zealand. Wellington, DSIR Publishing.